

The effect of hormonal stimulation of pigmentation upon the ability of the stimulated skin to form dopa-melanin was investigated, using both the pigmented nipple of the guinea pig and the hamster pigment spot. In both instances, tissues which had been caused to hypertrophy and blacken under hormonal influence gave increased color formation with dopa as contrasted with untreated control tissues, but the essential biphasic shape of the curves was unchanged.

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Discussion by MORRIS FOSTER,
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Two important sources of error are inherent in the techniques used: (1) The colorimetric method, whereby pigment production rate is equated with oxidase activity. Such reasoning could be erroneous. For example, a reducing agent reacting with a substance produced after the oxidation of dopa could inhibit pigment production without affecting dopa oxidase activity; although, on the basis of colorimetric criteria, inhibition or lack of enzyme activity would be inferred. (2) The use of dopa as a substrate, since dopa can be oxidized via both the phenolase and cytochrome systems, whereas tyrosine is not affected by the latter. Thus enzymatic oxidation of dopa could be wholly or partly attributed to the cytochrome system rather than to the phenolase system. (See paper by Hesselback et al.)

A more direct measure of phenolase activity could be obtained by using tyrosine as the substrate and by measuring the rate of oxygen consumption in a Warburg respirometer.

In regard to the hamster pigment spot, an explanation of increasingly inhibited oxidative activity with increasing weight of pig-

ment spot sample is difficult to make, since larger samples should also give larger amounts of enzyme, resulting in increased rather than decreased activity.

Reply by Shrader & Pfeiffer.

We would like to strongly emphasize that we do not know the enzyme system or systems involved in the production of dopa-melanin in these experiments. Our experiments were not designed primarily to elucidate the mechanisms of melanin formation but were intended to explain why the dopa reaction could not be obtained on sectioned hamster pigment spot. What we measured was the amount of dopa-melanin that was produced under experimental conditions as similar as possible to those of histological methods. The use of tyrosine as a substrate, or the Warburg apparatus as a method, would not have served to answer our original question. We do, however, feel that the results obtained justify the assumption that the colorimetric measurement of dopa-melanin is a valid criteria for an estimation of the pigment forming ability of the tissue.

In regard to the increase in inhibition which occurs as hamster pigment spot tissue weight is increased, we can only say that this is a characteristic of all skins tested and that we have postulated that it represents the results of some type of competitive action between the enzyme and inhibitor systems for utilization of the substrate. This suggested explanation gains further support from the fact that such inhibition is more marked when the quantity of available substrate is low and can be overcome by the addition of more substrate.

Fourth Session: Biochemical and Biophysical.

Introduction.

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Melanin chemistry may be roughly divided into two general approaches: (a) the character of the pigment noted in natural sources, i.e., the intracellular granules in skin, hair, plants, etc., and (b) the nature of the polymeric pigments obtained by the *in vitro* oxidation of such amino acids as tyrosine and dihydroxyphenylalanine, and the relation, if not the identity, of such pigments to those noted in natural sources. As yet, no clearcut

evidence has linked the synthetic pigments under (b) with the pigments derived from natural sources, although the presence of dopa oxidase in melanin-containing melano-blasts, and the absence of this enzyme from amelanotic tissues are more than suggestive of a connection between the two.

The natural pigment is generally associated with protein in the form of cytoplasmic granules. These granules themselves contain dopa oxidase activity, and an essential problem which requires clarification is whether the pigment may be preformed from small molecular weight precursors followed by conjugation with one or more proteins in the cytoplasmic complex or whether it is derived from certain potentially chromophoric groups (tyrosine residues) existing at the surface of the protein molecules of the complex. It is a matter of some difficulty to free the pigment completely of protein, and even when this is accomplished the vigorous methods required raise some doubt as to whether the melanin has not been altered to some extent. Indeed, the characterization of the particulate, sub-unit components of any cell, and the separation of the various members of these particulate components, demand techniques which for the most part are not yet adequately developed. However, advances in this, as in other fields, may come from many able quarters. That such advances have been made in several approaches to the problem of pigment chemistry are shown in the excellent papers which follow.

Mammalian Melanin Formation: I. Biochemical Studies.

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Investigation of the biochemistry of melanin formation in plants, insects and marine animals has shown that the enzyme, tyrosinase, catalyzes the oxidation of L-tyrosine to dihydroxyphenyl-L-alanine (dopa), and then the oxidation of dopa to melanin. Until recently, however, the presence of tyrosinase in mammalian tissue had not been demonstrated conclusively, and it was believed that melanin in mammalian tissue is formed by a

mechanism different from that operating in other species. Histochemical evidence indicated that mammalian skin contains an enzyme, "dopa oxidase," which catalyzes the oxidation of dopa, but not tyrosine, to melanin. Largely as a result of these beliefs, two separate hypotheses of melanogenesis evolved: 1) melanin formation in insects and plants was associated with tyrosinase, while 2) melanin production in mammalian skin was associated with dopa oxidase. Recent evidence has shown that these separate concepts can now be merged into a single hypothesis to account for melanin formation in man, lower animals, insects and plants (1). It is now known that the original distinction between tyrosinase and dopa oxidase is no longer valid. Hence, it is suggested that the single term, tyrosinase, should be used instead of the separate terms, tyrosinase and dopa oxidase (2).

While tyrosinase obtained from different species has some unique properties, depending on the particular source, three characteristics are common to tyrosinase, under proper conditions, regardless of its origin: 1) all catalyze the oxidation of tyrosine to melanin, 2) the enzymatic reaction with the monohydroxyphenyl compound is catalyzed by some orthodihydroxyphenyl compound (dopa, catechol, etc.), and 3) copper is associated with the activity of the enzyme.

When tyrosine and tyrosinase are allowed to react in the presence of oxygen, there is often a lag period before oxidation of tyrosine begins. This lag interval is referred to as the "induction period." Small amounts of dopa are very effective in shortening the induction period in the tyrosine-tyrosinase reaction. For mammalian tyrosinase there is a linear relationship between the negative logarithm of the dopa concentration and the induction period. Compounds related structurally to dopa, such as epinephrine, catechol and the like can shorten the induction period, but not nearly so effectively as dopa does. When dopa itself is used as a substitute for tyrosine, there is no induction period.

Dopa participates in the tyrosine-tyrosinase reaction in at least three ways: 1) dopa is formed from tyrosine, 2) dopa catalyzes the tyrosine-tyrosinase reaction, and 3) some amount of dopa is reformed during the conversion of dopa to melanin.

Various substances inhibit melanin formation *in vitro* and *in vivo*. The mechanism of inhibition is dependent upon the particular step in the tyrosinase-catalyzed series of reactions in which the conversion of tyrosine to dopa and eventually to melanin is blocked.

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